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SEARCH FOR OPTICAL ACTIVITY IN THE
ORGUEIL METEORITE

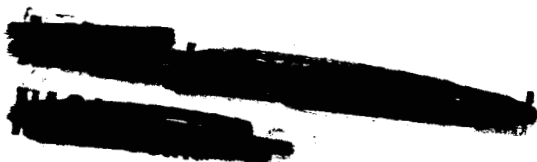
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Nagy et al¹ have reported the discovery of optical activity in the Orgueil carbonaceous chondrite. They observed a rotation of $-0.023^{\circ} \pm 0.005^{\circ}$ in an extract consisting mainly of organic acids and hydrocarbons, and concluded: "It seems reasonable to connect optical rotation in Orgueil with biological activity, either of indigenous origin or perhaps still related to terrestrial contaminations."

In view of the far-reaching implications of this result, I decided to verify the findings of Nagy et al.

Orgueil Meteorite. Through the courtesy of A. Cavaillé, I obtained a freshly broken interior sample of a 682-gram stone from the Natural History Museum, Montauban, France. The sample was completely free of fusion crust and any visible surface contamination.

Extraction and Saponification.² The sample of Nagy et al was chemically heterogeneous, consisting of hydrocarbons and sulfur in addition to organic acids. It seemed desirable to separate

these components from each other, not only to lessen the danger of mutual cancellation of optical activity, but also to avoid interference by sulfur, which had been a problem in the work of Nagy et al. I therefore modified their procedure slightly, to eliminate sulfur and to separate hydrocarbons from acidic compounds.

Sample A. Twenty grams of finely powdered meteorite was put through three Soxhlet extractions with 100 ml. each of benzene, chloroform, and methanol. After evaporation of the solvents under reduced pressure, the yellow residue was completely dissolved in a boiling benzene-ether mixture, which was then kept at 0° C for 24 hours. Much of the sulfur precipitated on standing. It was removed by filtration and washed with ether and n-hexane. [To rule out the remote possibility that a small amount of an optically active compound was lost with the sulfur, I dissolved the residue in benzene, and passed the solution through a colloidal copper column. Evaporation of the now sulfur-free solution gave a minute residue of 0.26 mg (sample A1).] After evaporation of the solvent, the pale yellow residue was saponified by refluxing it for three hours with methanolic alkali (1 gram KOH in 12 ml methanol and 3 ml water). The solution was poured into 60 ml of water, with constant stirring, and extracted with ether to remove the unsaponifiable material, e.g. hydrocarbons and traces of sulfur.

The unsaponifiable fraction was purified according to the method of Blumer³ to remove the sulfur. It yielded 24.45 mg of an oily solid (sample A2). The aqueous solution was acidified with 10 per cent HCl and extracted with 60 ml of ether in a liquid/liquid

extractor for a period of twelve hours. The ether solution was washed with water and dried, and the solvent was removed. The residue, sample A3, weighed 10.76 mg.

Sample B. 16 grams of finely powdered Orgueil meteorite which had already been extracted with water and a variety of organic solvents⁴ was hydrolyzed with 10 per cent HCl for 10 hours at 100° C. The hydrolyzate was filtered to remove the insoluble residue and extracted with ether five times. The insoluble residue was treated with ether for three hours at refluxing temperature, the ether solution was filtered, and combined with the ethereal solution obtained above. The combined solution was washed with water, dried, and evaporated under reduced pressure. The residue consisted of 9.63 mg of a pale yellow oil (sample B).

Methyl esters of A3 and B. To obtain another set of chemically well-defined samples, I treated samples A3 and B with diazomethane, thereby converting the fatty acids to their methyl esters (samples MA3 and MB). The ester samples, weighing 7.07 and 7.37 mg, were colorless. They were purified by chromatography through an alumina column.

Infrared and ultraviolet spectrophotometry. The principal absorption maxima of samples A2, A3, B and their methyl esters are listed in Table 1. The data of Nagy et al are given for comparison. Clearly, there is excellent agreement between the IR spectra of my samples A3 and B and Nagy's sample. All three, in turn, match the IR spectra of long-chain, saturated carboxylic acids. This identification is confirmed by the spectra of the methyl esters. The strong triplet absorption at 8.01, 8.34 and 8.56 μ is a characteristic feature of the spectra of methyl esters

of long chain acids.⁵ The methyl ester of sample B seemed to consist largely of saturated acid esters, with a small admixture of non-conjugated unsaturated acid esters.^{6,7} The presence of unsaturated esters in sample B (and their absence in sample A3) was confirmed by thin-layer chromatography, using the method of Mangold et al.⁸

Three compositional differences between my samples and those of Nagy et al should be noted. Nagy et al attached some significance to the absorption bands at 262 and 277 μ which were present in their samples but not in recent biological material (Figure 1). These bands, which were absent from my samples, seem to have been due to elemental sulfur.^{9,10,11} Figure 1 shows a spectrum of pure sulfur along with spectra of Nagy's Orgueil extract and my samples A2 and A3. Most of the spectral features of Nagy's sample, including the peaks at ≈ 262 and ≈ 277 μ , also appear in the sulfur spectrum. The Orgueil meteorite contains between 1 and 2 per cent free sulfur, and it seems that this element, rather than some hypothetical organic compound, was responsible for the extra absorptions seen by Nagy et al.

It appears that yet another type of extraneous material was present in the samples of Nagy et al: organic sulfur compounds produced during saponification by reaction of benzene, methanol, and meteorite organics with free sulfur and KOH. A control experiment duplicating Nagy's saponification conditions showed that 260 mg of sulfur refluxed for five hours with 100 ml of a benzene-

methanol mixture (6:4) in the presence of 2.9 g of KOH and 1 ml of water evolved H_2S and left a residue of 93 mg of organic sulfur compounds and sulfur. A typical 22-gram sample of Orgueil contains about 400 mg of S, and since Nagy's extract contained a number of meteorite organics in addition to benzene and methanol, an even greater variety of such "secondary" reaction products might be expected. This is an important point, to which we shall return later.

A final compositional difference concerns hydrocarbons and other non-saponifiable compounds, which comprised a substantial part of Nagy's sample. Owing to the lack of distinctive spectral features, they did not contribute significantly to his UV and IR spectra, but were detected by thin-layer chromatography. In my procedure, these compounds appeared largely in sample A2, which was investigated separately. Together, my samples A1, A2, and A3 were equivalent to the samples of Nagy et al.

Controls. The reported optical rotation, though appreciably greater than the nominal sensitivity of the polarimeter, lies in a range where instrumental artifacts can become serious. To guard against such artifacts, three optically inactive controls were prepared. Control 1 was a saturated solution of sulfur in $CHCl_3$. Control 2 was essentially a blank for the procedure of Nagy et al: a chloroform solution of the mixture of sulfur and sulfur compounds prepared by refluxing S and KOH with benzene and methanol (see above). Control 3 was a dark yellow solution of impure acylated melamine.

Optical Rotation. To duplicate the conditions of Nagy et al as closely as possible, my measurements were likewise performed on three different Rudolph polarimeters at three different institutions: a photoelectric polarimeter with oscillating polarizer at the University of Chicago; a recording spectropolarimeter at the University of Alberta; and an automatic spectropolarimeter at the Argonne National Laboratory. For the sake of brevity, only the results with the last-named instrument are cited, but the other two sets of data were quite similar (Table 2).

None of the six samples investigated showed a significant optical rotation at 440 m μ . The highest result, -0.00024° , was nearly an order of magnitude lower than the rotation of $-0.023^\circ \pm 0.005$ and $-0.020^\circ \pm 0.005$ which Nagy et al found in two samples of Orgueil, at 435 and 440 m μ , respectively. Moreover, two of my controls gave higher values than any of my meteorite samples: -0.0053° and -0.0093° .

Nagy et al had noted that the optical rotation of their samples increased at lower wavelengths, but, owing to the presence of sulfur and its secondary reaction products, they were unable to go below 435 m μ . Neither of these interfering substances was present in my samples, and I was therefore able to extend the measurements down to 340 m μ . As the transparency limit was approached, the samples began to show an increasing "rotation", but since the controls showed the same behavior, this "rotation" seems to have been an instrumental artifact arising under conditions of low light transmission. For ease of comparison, the

data are plotted in Figure 2. Note that at 340 m μ the transparent sample MA3 gave only one-tenth the rotation of the rather strongly absorbing sample A3, although both were of similar composition and concentration. The controls, all of which had appreciable absorption in the near UV, gave even larger readings at this wavelength, which, however, fluctuated badly. It would seem that strongly absorbing solutions are apt to give a false laevo-rotation.

Figure 2 also includes Nagy's data on Orgueil. Evidently, my samples do not show the conspicuous laevo-rotation at 440 m μ , which is present in Nagy's sample. Before accepting this discrepancy as significant, we must examine some of the more obvious sources of error. At least four possibilities come to mind.

(1) Chemical Composition; i.e., the optically active compounds were lost in my procedure. This is hardly possible. Both procedures were based on the same principles: solubility in benzene and methanol, saponifiability, solubility in ether. All material initially extracted from the meteorite was measured on the polarimeter. The IR and UV spectra, and the thin-layer chromatograms show that all classes of organic compounds identified by Nagy et al were also present in my samples.

(2) Sample Size. Nagy et al did not give the weight of their samples, owing to the presence of an indeterminate amount of sulfur. But there is good reason to believe that my samples were of comparable size. Nagy et al used the extraction procedure of Nagy and Bitz^{1,2} which had previously yielded 580-675 μ g of carboxylic acid methyl esters per gram of Orgueil. This corresponds

to (calculated) ester contents of 14.9, 7.8, and 7.5 mg for the three samples of Nagy et al. My ester samples initially were of similar size: 7.1 and 7.4 mg, although repeated transfers eventually reduced these amounts to 6.4 and 6.6 mg.

(3) Inhomogeneity of Meteorite. One might speculate that the optically active organic compounds are very unevenly distributed among different stones of the Orgueil meteorite shower, and that my 682-gram stone happened to contain much lower levels of these compounds than did the three stones of Nagy et al. This is improbable. As shown above, the 682-gram stone contains comparable amounts of carboxylic acids; it also contains perfectly normal levels of amino acids,^{1,3,14} nitrogen compounds,¹⁵ hydrocarbons^{16,17} sulfur, epsomite, and many organic compounds (R. Hayatsu, unpublished work).

(4) Instrumental Sensitivity The polarimeters used in this study had sensitivities of 0.001° and 0.0005° . This corresponds to 4 per cent and 2 per cent the rotation reported by Nagy et al, $-0.023^\circ \pm 0.005$. Apparently these instruments were sensitive enough to detect an optical rotation of the order of 0.02 degrees. Of course, the over-all sensitivity also depends on the cell length, cell volume, and concentration. My measurements were performed in semi-micro cells of 1 dm path length and 0.7 ml volume, at concentrations of 6-12 mg/ml. Nagy et al state neither the concentration, nor the cell volume or cell length used in their measurements. They mention that their data were recalculated to some standard cell length, but do not state explicitly what this cell length was. Even though it is not

possible, for lack of information, to determine the exact sensitivity of Nagy's measurements, there seems to be no indication that it was significantly higher than that of the present study. Solubility and optical transmittancy considerations alone preclude appreciably smaller cell volumes and higher concentrations.

Thus a serious discrepancy exists between the results of the present study and those of Nagy et al. The cause of the discrepancy is not entirely clear. Nagy et al concede that the laevo-rotation detected by them might "perhaps still [be] related to terrestrial contamination." Another possibility is interference by colloidal sulfur, which was intentionally put in some of their blanks, apparently to compensate for the colloidal sulfur content of the samples. Finely dispersed particulate matter can simulate a false optical rotation. A slightly turbid solution of my sample B gave an apparent laevo-rotation of as much as -0.08° which disappeared completely after the solution was filtered. Nagy et al filtered two of their three samples through a 0.45μ ultra-filter, but a filter of this pore size cannot remove colloidal particles.

It is perhaps very significant that the optical rotation found by Nagy et al always appeared very close to the absorption cutoff (Table 3). Instrumental artifacts are most likely to arise under conditions of low light intensity; indeed, even my optically inactive controls gave an apparent "rotation" in regions of appreciable absorption. Control 2 is especially pertinent to this question. It consisted largely of organic sulfur compounds derived from the reagents in Nagy's saponification procedure. An aliquot of 10 mg sufficed to give an apparent optical rotation of -0.0053°

at 440 μ : one-fifth that reported by Nagy in Orgueil. But the total amount produced under conditions duplicating Nagy's procedure was 93 mg; more than enough to account for the observed rotation.

To some extent, the effect of light absorption can be compensated by use of colored blanks. But Nagy's blanks had a lower absorbance at 440 μ than did the meteorite extract (Figure 2 in Nagy et al). This uncompensated light absorption may well have been responsible for most or all of the apparent rotation.

Finally, a few comments should be made on the quantitative significance of the negative results obtained in the present study. The highest apparent "rotation", -0.0024° at 440 μ , was obtained on sample A3. This corresponds to a specific rotation $[\alpha]_{440}$ of $\leq 0.33^\circ \text{ ml g}^{-1} \text{ dm}^{-1}$. Most optically active carboxylic acids have larger specific rotations, the order of $10^\circ \text{ ml g}^{-1} \text{ dm}^{-1}$. But in a sample containing a mixture of acids, mutual cancellation of activity and dilution by inactive acids will depress this value greatly. Indeed, neither the fatty acids from brown algae nor the naphthenic acids from petroleum measured by Nagy et al gave any detectable rotation in spite of their evident (or presumed) biological origin. Perhaps the most conclusive result to date has been obtained on an amino acid fraction from Orgueil¹³. Here, the mutual cancellation of activity was explicitly taken into account. If these amino acids were biogenic, and consisted of one optical isomer only, a rotation of 0.0046° would have been expected. The value actually obtained was $\leq 0.001^\circ$.

Summary: This work does not confirm the reported presence of optically active compounds in the Orgueil meteorite. None of the several fatty acid and hydrocarbon fractions measured gave a rotation exceeding that of optically inactive controls. The highest value observed, -0.0024° , is an order of magnitude lower than the rotation reported by Nagy et al, -0.023° . It corresponds to a specific rotation $[\alpha]_{440}$ of less than 0.33° ml g⁻¹dm⁻¹.

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¹⁷ Meinschein, W. G., Nagy, B., and Hennessy, D. J., Ann. N. Y. Acad. Sci., 108, 553 (1963).

TABLE 1

I.R. and U.V. Spectral Data of Orgueil Meteorite Extracts

Author	Sample	I.R. (μ)	U.V. (m μ)
Nagy et al	1, 2 ^{a)}	3.3, 3.7, 5.85, 10.7	220, 262, 277
Hayatsu	A3 ^{b)}	3.34, 3.69, 5.85, 10.69	218
Hayatsu	B ^{b)}	3.34, 3.68, 5.84, 5.89, ^{d)} 10.68	218-220
Hayatsu	MA3 ^{b)}	5.75, 8.01, 8.34, 8.56	Not measured
Hayatsu	MB ^{b)}	5.77, 5.81, ^{d)} 8.0-8.1, 8.39, 8.57	Not measured
Hayatsu	A2 ^{c)}	6.11-6.34 ^{e)}	No absorption between 220-700

I.R. spectra were measured in:

a) CCl_4 ; b) CS_2 ; c) CHCl_3 . All U.V. spectra were measured in methanol. d) weak shoulder; e) weak broad absorption.

TABLE 2

Optical Rotation of Orgueil Meteorite Extracts and Controls*

Sample	Concentration mg/ml	Rotation (degrees) at wavelength (mμ)					
		560	500	440	400	360	340
A1	0.26/1 ml	-0.0012	--	±0.000	--0.0010	--	--
A2	12.65/1 ml	-0.0011	-0.0010	-0.0013	--0.0036	-0.0073	-0.020
A3	7.21/1 ml	-0.0010	-0.0030	-0.0024	--0.0029	-0.0061	-0.039
B	8.40/1 ml	-0.0016	-0.0014	-0.0018	--0.0024	-0.0080	--
MA3	6.35/1 ml	-0.0010	+0.0012	-0.0010	--0.0013	-0.0030	-0.0043
MB	6.62/1 ml	-0.0016	--	-0.0016	--0.0022	--	-0.0040
Control 1	Saturated	-0.0015	-0.0013	-0.0018	--0.0050	-0.0096	--
Control 2	10.02/1.4 ml	-0.0014	-0.0039	-0.0053	--0.0092	+	+
Control 3	10.17/1.4 ml	-0.0020	-0.0021	-0.0093	--0.016	+	+

*This series of measurements was performed on a Rudolph Automatic Spectropolarimeter with a sensitivity of 0.001°. Cell length: 1 dm; cell volume 0.7 ml, except for Control 1 (8 ml); and Controls 2, 3 (1.2 ml).

Symbols: - Not measured; + observed "rotation" large, but irreproducible, owing to strong light absorption by sample.

TABLE 3

Optical Rotation of Orgueil Samples of Nagy et al

	Sample 1	Sample 2	Sample 3
Maximum rotation (degrees)	-0.020	-0.012	-0.023
λ of maximum rotation (m μ)	440	450	435
λ of "no transmittance" (m μ)	435	440	406

FIGURE CAPTIONS

Fig. 1. Ultraviolet absorption spectra of Orgueil meteorite extracts. Unidentified features at 262 and 277 $m\mu$ in sample of Nagy et al seem to be due to elemental sulfur, rather than to organic compounds.

Fig. 2. Optical rotation of Orgueil meteorite extracts and controls. Meteorite extracts prepared in this work do not show a significant laevo-rotation at 435-440 $m\mu$, in contrast to sample of Nagy et al. However, both the extracts and optically inactive control samples begin to show a false "rotation" as their absorption cutoff is approached. "Rotation" in sample of Nagy et al may be of the same spurious origin since it was measured only 5 $m\mu$ above the absorption cutoff.

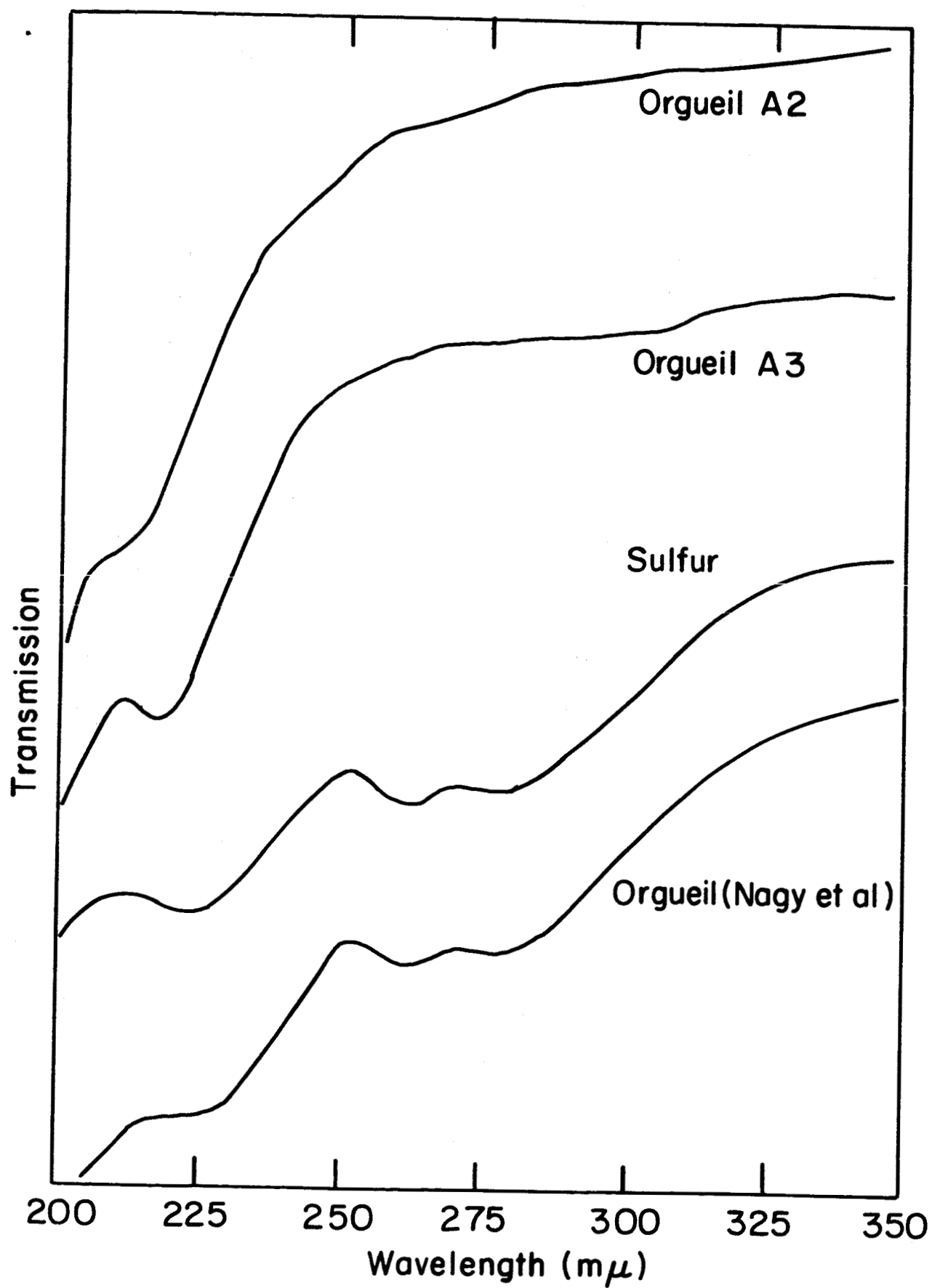


Fig.1

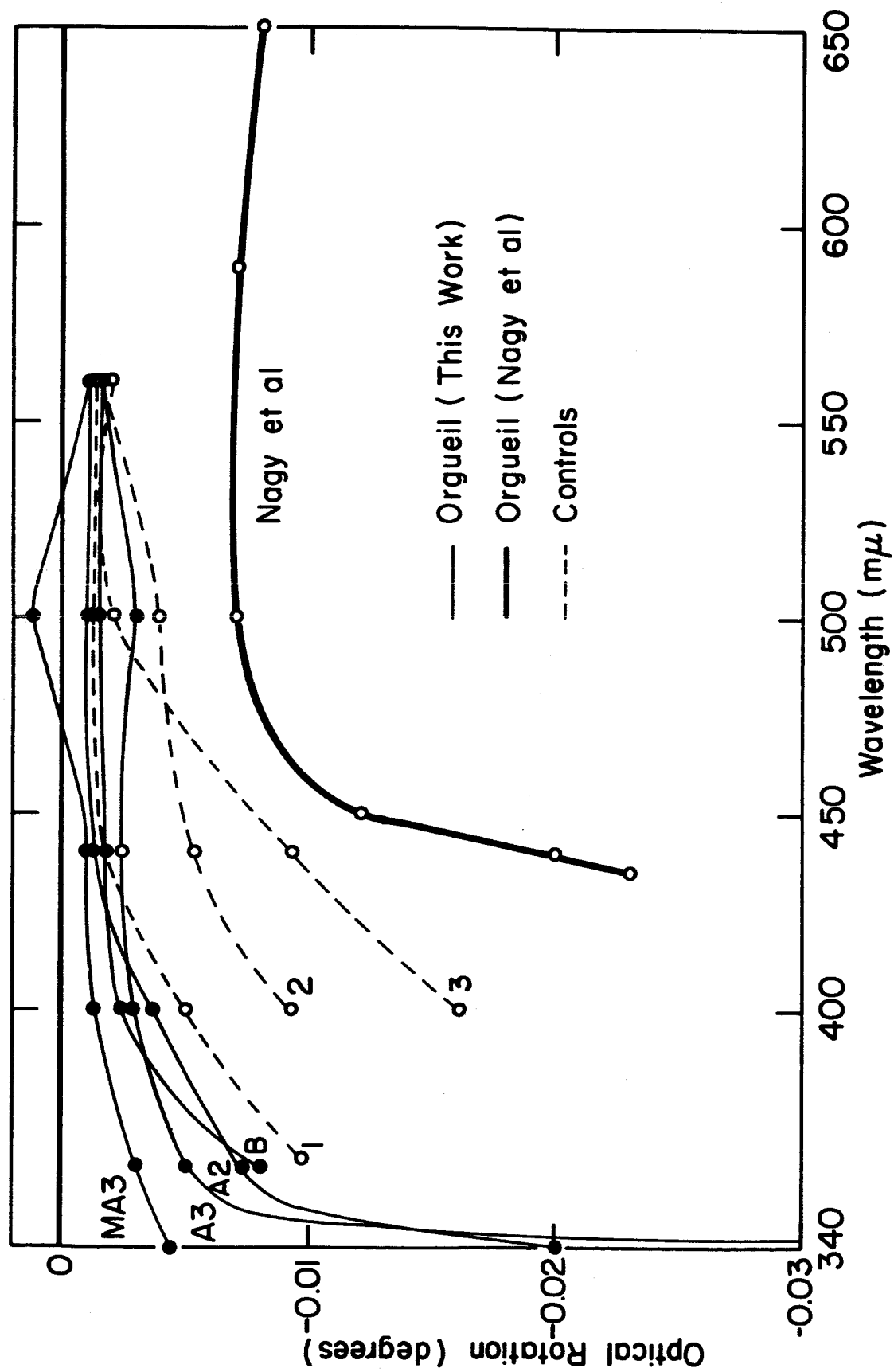


Fig.2